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Microalgae as a boon for sustainable energy production and its future research & development aspects

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ABSTRACT

Increasing energy demands, predicted fossil fuels shortage in the near future, and environmental concerns such as the production of greenhouse gas carbon dioxide have motivated the search for alternative and cleaner energy sources. Biodiesel has received much attention in recent years and production of biodiesel from microalgae is a newly emerging field. Microalgae possess a high growth rate, utilize solar light, water and CO_2 to convert these to sugars, from which macromolecules, such as lipids and triacylglycerols (TAGs) can be obtained making microalgae more photosynthetically efficient than oil crops. Microalgae are represented as a potential source of biomass, having great biodiversity and variability in their biochemical composition. This paper presents an overview on microalgae with particular emphasis as a source for biofuel and its other applications. Future research and development aspects regarding microalgae and microalgal fuel production are also discussed.

Contents

1. Introduction

Progress in scientific knowledge and its application via industrial products have improved living standards and has also lead to additional questions, concerns, and speculations from citizens

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or opinion leaders. Technological improvements have both reduced as well as increased the production risk. Furthermore, the effects of technological change on production risk have varied over time, space, and production activities. A major global challenge faced today is the increasing price of food, feed associated, in part at least, with alternative uses of food, and feed crops in biofuel production. The reported current consumption of petroleum is at 10^5 times faster than nature can create [1]. These facts, along with the limited resources of oil reserves (stocks of fossil fuels) and its use contributing to the increase of atmospheric CO_2 leading to global warming [2] are currently recognized as great threats to mankind. In order to resolve the worldwide energy shortage crisis, seeking for lipid-rich biological materials to produce biodiesel effectively has attracted much renewed interest. Demanding energy requirement and the ecological considerations have led to finding substitutes for the fossil fuels by other resources including renewable sources derived from biologically based fuels such as biomass and biofuels [3], which have attracted increased attention as evident from the growing literature. Biofuels have become increasingly necessary for the global fuel market and are produced from vegetable oils, recycled cooking fats or waste oils, animal fats, or microalgal lipids [4] and is known to mankind since ancient days. Biodiesel appears to be an attractive energy source for several reasons. First, biodiesel is a renewable resource of energy that could be sustainably supplied as the petroleum reserves are to be depleted in less than 50 years at the present rate of consumption [5]. Second, biodiesel have several favorable environmental properties like less release of carbon dioxide, sulfur content and carbon monoxide [6]. The release of sulfur content and carbon monoxide would be cut down by 30% and 10%, respectively, by using biodiesel as reported in the literature. Biodiesel contains no aromatic compounds and other chemical substances which are harmful to the environment and human health. Third, biodiesel appears to have significant economic potential because as a non-renewable fuel that fossil fuel prices will increase inescapability further in the future [7]. Finally, biodiesel is better than diesel fuel in terms of flash point and biodegradability [8]. Conventional biodiesel mainly is produced from soybean and vegetable oils, palm oil, sunflower oil, rapeseed oil as well as restaurant waste oil. Table 1 lists some of the renewable sources of biodiesel including microalgae. From this table it is clearly indicated that substitution of the diesel used in the transport sector by the biodiesel produced from cultivated plants would need the use of massive lands that are presently used to produce food [9]. In recent years, several countries have embarked on legislative and regulatory pathways that encourage increased use of biodiesel fuel-using both incentives and prescriptive volumetric requirements. For example, in U.S., the Energy Independence and Security Act (EISA) of 2007 established a 0.5 billion gallon/year (bg/y) requirement for biomass-based diesel fuel in 2009, that has increased to 1.0 bg/y by 2012 [10].

Table 1
Oil yield of sources of biodiesel [2].

Source	Yield of oil ($L\ ha^{-1}$)	Required land area (Mha)*
Corn	172	1540
Soyabean	446	594
Canola	1,190	223
Jatropha	1,892	140
Coconut	2,689	99
Oil palm	5,950	45
Microalgae**	70,405	7.6
Microalgae***	35,202	15.2

* To meet 50% of all transport fuel needs of U.S.A.
** 40% oil (% dry wt) in biomass.
*** 20% oil (% dry wt) in biomass.

Using biofuels in a managed fashion is the professed policy of governments, and for this a strong agriculture sector must be developed that is both productive and environment-friendly. Biodiesel derived from oil crops cannot realistically meet existing costs of higher fraction of the raw materials and competitive demand of the soil for their growth [4] as the cost of raw material accounts 50–85% for the total production cost [11]. So, the cost of material is the dominant factor in fixing the price of biodiesel. There are numerous criticisms for such promotion of lands for renewable source of energy [12] and also arguments for and against the biofuels from microalgae and plant resources [2,3]. Though there might be large amounts of low-cost oil and fats available such as restaurant waste and animal fats [13], the major problem of using these low-cost oils and fats is the occurrence of large amounts of free fatty acids (FFA) which is difficult to convert into biodiesel through transesterification [14]. So, raw materials containing large proportions of fatty acid triglycerides are preferred. Microalgae are the promising source for the production of biofuels as they utilize carbon dioxide, sun light and carbon source. Also, they have higher photosynthetic efficiency than

Table 2
Different sources for the oils production and their comparison [18].

Type of organism	Advantages	Disadvantages
Microalgal oils	(1) Fatty acid constitutions similar to common vegetable oils (2) Under certain condition it may be as high as 85% of the dry weight (3) Short-time growth cycle (4) Composition is relative single in microalgae	(1) Most algal lipids have lower fuel value than diesel fuel (2) The cost of cultivation is higher compared to common crop oils currently
Bacteria oils	(1) Fast growth rate	(1) Most of bacteria can not yield lipids but complicated lipoid
Oleaginous yeasts and mildews	(1) Resources are abundant in the nature (2) High oil content in some species (3) Short-time growth cycle (4) Strong capability of growth in different cultivation on conditions (5) Conversion and utilization of scrap fiber to yield useful oils and the application for dealing with waste oils in environment	(1) Filtration and cultivation of yeasts and mildews with high-content oils are required (2) Process of oils extracted from oleaginous yeasts and mildew is complex and new technology should be exploited to resolve it (3) The cost of cultivation is also higher compared to common crop oils currently
Waste oils	(1) The waste oils is cheap compared to crop oils	(1) Containing a lot of saturated fatty acids which is hard to be converted to biodiesel by catalyst

terrestrial plants and are efficient carbon dioxide fixers. Therefore, higher biomass productions along with faster growth rate over energy crops [4,15,16] are observed. Microalgae have been estimated to have higher biomass productivity than plant crops in terms of land area required for cultivation, are predicted to have lower cost per yield, and have the potential to reduce GHG emissions through the replacement of fossil fuels [17]. Using corn as a feedstock for making ethanol creates a negative competition between human and animal consumption or fuel production [18]. Microalgae also have the potential to counteract a portion of the greenhouse effect and water pollution, because through photosynthesis, microalgae have the ability to fix carbon dioxide released by industries in the environment. Some microalgae also fix nitrogen and absorb other contaminants such as heavy metals and phosphorous [19,20]. Oleaginous microorganisms are favorably considered for their short growth cycles, high lipid contents and ease of being modified by biotechnological means (Table 2).

Additionally, high production of biomass and some metabolites are achieved by their heterotrophic growth [21–23]. Depending on higher photosynthetic efficiency and other characteristics mentioned above, microalgae would have cost advantage as oil content of several microalgae species might reach up to 80% of its dry weight and their productivity can be enhanced by genetic manipulations making microalgal biodiesel economically competitive with petrodiesel through large-scale production [4].

Microalgae as a renewable source for obtaining fuel is an old concept proposed in fifties with follow up in sixties and seventies particularly for producing biogas [24] and later reported for liquid fuel in eighties and nineties [2,3] and have received increased attention in recent because of increasing petroleum prices and ecological considerations [25]. Also, this seems to be the ideal solution for total substitution of the diesel used in the transport [2]. Means of ensuring satisfactory in-use biodiesel fuel quality is primarily establishment of a rigorous set of fuel specifications, such as American Society for Testing Materials (ASTM) D6751 (in the U.S.) and European standard (EN) 14214 (in the European Union). Other countries have defined their own standards and many of these standards are derived from either ASTM D6751 or EN 14214. Some countries have also worked together to define guidelines for regional biodiesel standards [10]. For example, a group called the Asia-Pacific Economic Cooperation (APEC) issued a report in 2007 that addressed guidelines for standardizing biodiesel standards within the APEC region [26]. According to biodiesel standard published by the ASTM, biodiesel from microalgal oil is similar in properties to the standard biodiesel, and is also more stable according to their flash point values (Table 3). Recently, review by Hoekman et al. highlighted that the biodiesel produced from *Chlorella protothecoides* satisfied several of the ASTM specifications for biodiesel. This review also highlighted that biodiesel produced from six different algal species meets several of the specifications matching European biodiesel [10].

Considering the above facts, this paper gives an overview on microalgae as a source for biodiesel, energy and new materials. Production of microalgae, microalgal oils their characteristics, applications in various areas and future research and development aspects are presented.

2. Biodiesel

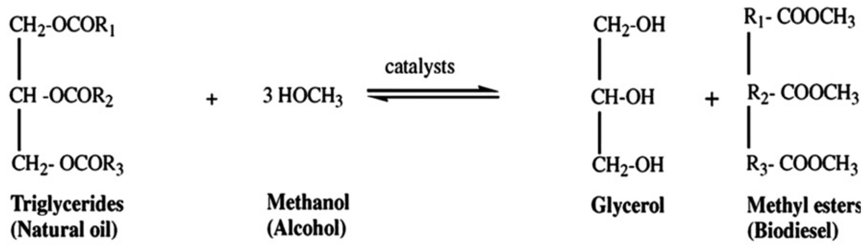
Biodiesel is a proven fuel and its production technology has been known for more than 50 years [2,27,28]. Biodiesel is the monoalkyl esters of long-chain fatty acids derived from renewable feedstocks, such as vegetable oil or animal fats [27] and is also non-toxic and biodegradable [28]. Oilseed crops such as rapeseed and soybean oil have been extensively evaluated as sources of biodiesel. Biodiesel is quite similar to conventional diesel fuel in its manufacturing and it can be used in existing diesel engines without any modification, and can be blended in any ratio with conventional petroleum and diesel fuel [9].

2.1. Biodiesel chemistry and transesterification

Plant oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities and therefore cannot be used as fuel directly. These problems can be sorted out by slight chemical modification mainly transesterification, pyrolysis and emulsification. Among these, the transesterification is the key and foremost important step to produce the cleaner and environmentally safe fuel from vegetable oils [27]. Natural oil present in the oilseeds is generally in the form of TAGs [29]. TAGs consist of three long chains of fatty acids attached to a glycerol backbone. By reacting these TAGs with simple alcohols (a chemical reaction known as "transesterification"), a chemical compound known as an alkyl ester is formed, which is known more generically as biodiesel (Fig. 1). Various methods are available for the extraction of algal oil, such as mechanical extraction using hydraulic or screw, enzymatic extraction, chemical extraction through different organic solvents, ultrasonic extraction, and supercritical extraction using carbon dioxide above its standard temperature and pressure. In enzymatic extraction water is used as solvent with the cell wall degrading enzymes for fractionation of oil, proteins and hulls and oil is found inside plant cells, linked with proteins and a wide range of carbohydrates like starch, cellulose, hemi-cellulose and pectin [30]. Protein and oil are released by the opening of thick cell wall by enzymatic degradation, down-stream processing making fractionation of the components possible to a degree which cannot be reached when using the conventional technique like mechanical pressing and its the biggest advantage of enzymatic extraction. Cost of this extraction process is much higher than most popularly used solvent based extraction processes [30]. The high cost of extraction serves as a limitation factor for large scale utilization of this process. The Soxhlet method is the most commonly used solvent extraction method for the extraction of oil from various

Table 3
Comparison of properties of microalgal oil, conventional diesel fuel, and ASTM biodiesel standard [16].

Properties	Biodiesel from microalgal oil	Diesel fuel	ASTM biodiesel standard
Density (kg L^{-1})	0.864	0.838	0.84–0.90
Viscosity ($mm^2 s^{-1}$, cSt at 40 °C)	5.2	1.9–4.1	3.5–5.0
Flash point (°C)	115	75	Min 100
Solidifying point (°C)	−12	−50 to 10	–
Cold filter plugging point (°C)	−11	−3.0 (max −6.7)	Summer max 0 Winter max < −1.5
Acid value (mg KOH g^{-1})	0.374	Max 0.5	Max 0.5
Heating value (MJ kg^{-1})	41	40–45	–
H/C ratio	1.81	1.81	–



Fatty acid	No. of carbon : No. of double bond	Fatty acid chain
Palmitic	16:0	R = - $(CH_2)_{14}$ – CH_3
Stearic	18:0	R = - $(CH_2)_{16}$ – CH_3
Oleic	18:1	R = - $(CH_2)_7$ CH=CH $(CH_2)_7CH_3$
Linoleic	18:2	R=-$(CH_2)_7$CH=CH-CH_2-CH=CH $(CH_2)_4CH_3$
Linolenic	18:3	R= - $(CH_2)_7$ CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH

Fig. 1. Transesterification of triacylglycerols (TAGs) [130].

plants and algal strains. In the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washings with an organic solvent, usually *n*-hexane or petroleum ether, under reflux in a special glassware called Soxhlet extractor. Several advantages like large amount of extraction using limited solvent, cost effectiveness and can be used at large scale economically. Despite of these advantages there are certain limitations like, poor extraction of polar lipids, long time required for extraction, hazards of boiling solvents etc. Still it is the most popular and generally used in all oil extraction laboratories [31].

The ultrasonic extraction of algae oil involves intense sonication of liquid which generates sound waves that propagate into the liquid media resulting in alternating cycles of high and low-pressure. During the high pressure cycle the diffusion of solvents, like hexane takes place into the cell. By the mechanical cavitations shear forces, it facilitates the transfer of lipids from the cell into the solvent. Ultrasonication not only improves the extraction of oil from the algae cells but also helps its conversion to biodiesel [32]. The large scale application of this method is not feasible as it is not cost effective with the amount of oil production. In CO_2 supercritical method, CO_2 is compressed beyond its supercritical point (31 °C and 74 bar) where it obtains substantial solvent power. The supercritical fluid is brought in contact with algal material in an extraction vessel. Due to its high diffusion rates and gas like viscosity, CO_2 penetrates into the smallest pores of the starting material. In a separate vessel CO_2 is de-pressurized and substances of interest are efficiently collected with less solvent residues as compared to other extraction methods [25]. The CO_2 supercritical is the most advanced method, although disadvantages like elevated pressure requirement and high capital cost for equipment are associated with it and advantages associated are the biomass residues that remains after extraction of oil could be used partly as high-protein animal feed and, possibly, as a source of small amounts of other high-value microalgal products [33–35]. The algal biomass residue remains after oil extraction can also be used to produce biogas by anaerobic digestion. Which can further be used as the primary source of energy for most of the production and processing of the algal biomass. An additional income could come from the sale of nutrient-rich fertilizer and irrigation water that would be produced during the anaerobic digestion stage [36]. The technology for anaerobic digestion of waste biomass exists and is well developed [37], and the technology for converting biogas to electrical/mechanical power is well established [38]. The carbon dioxide generated from combustion of biogas can be recycled directly for the production of the microalgae biomass [19,22].

Transesterification is catalyzed by acids [29]; alkalis [27] and lipase enzymes [39]. Lipid extraction from microalgal biomass has not received sufficient attention and represents one of the many bottlenecks hindering economic industrial-scale production of microalgal biodiesel. Future research on microalgal biodiesel should focus on developing an effective and energetically efficient lipid extraction process. A fundamental understanding of lipid mass transfer mechanisms from the microalgal biomass to the extraction solvent is needed to scale up the lipid extraction process [40]. In order to achieve efficient transesterification reaction, the choice of catalyst is very important. The traditional liquid acid and alkali catalyst are called homogeneous catalysts because they act in the same liquid phase as the reaction mixture. Due to their simple usage and less time required for lipids conversion, the homogeneous catalysts dominate the biodiesel industry. However, the transesterification catalyzed by homogeneous catalysts needs high purity feedstock and complicated downstream processing [41], so high efficiency and low pollution catalysts such as solid acid & alkali catalysts, enzyme catalyst, supercritical catalyst systems and ionic liquid catalysts are receiving increasing attentions. Some scientists [42] studied the catalytic process using supercritical methanol and porous titania microspheres in a fixed bed reactor to catalyze the simultaneous transesterification and esterification of triglycerides and free fatty acids to biodiesel. The process was able to reach conversion efficiencies of up to 85%. Patil et al. reported a process involving simultaneous extraction and transesterification of wet algal biomass containing about 90% of water under supercritical methanol conditions [43].

Alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction [29]. Sodium and potassium hydroxide are commonly used alkalis as commercial catalysts at a concentration of about 1% by weight of oil whereas sodium methoxide (an alkoxide) are even better catalysts than sodium hydroxide and are being increasingly used. Use of lipases offers important advantages, but is not currently feasible because of the relatively high cost of the catalyst [29].

Alkali-catalyzed transesterification is carried out at approximately 60 °C under atmospheric pressure, as methanol boils at

65 °C at atmospheric pressure & in these conditions, tranesterification takes about 90 min to complete. A higher temperature along with higher pressure can be used but this is expensive. Methanol and oil do not mix; hence the reaction mixture contains two liquid phases. Other alcohols can be used, but methanol is the least expensive. The main drawback of alkali method is formation of soap in a reaction between free fatty acids present in oils extracted from algal biomass (glycerides, polar lipids and other solvent extractable molecules) and a base catalyst [44]. Alkali catalyst can be problematic when free fatty acid content in the oil is above 3% yielding it unsuitable for direct biodiesel production from unrefined oils [45]. The free fatty acid content of algae was found to be as high as 35.1% for some species, which is far beyond the limit recommended for alkaline catalysis [46]. Moreover, the reports of successful synthesis of microalgal biodiesel with alkali catalyst are difficult to compare as most of these studies lack conversion efficiencies and yields. Most studies generally employ the two step process with exception of work by Xu and Mi [47], where transesterification was performed in situ directly from dried biomass in the presence of a co-solvent-toluene. To prevent yield loss due to saponification reactions (i.e., soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol [2].

Acid catalyst catalyse reactions of fatty acid esterification and oil transesterification, thus increasing the overall theoretical yield of the reaction and allowing the use of feedstocks containing higher free fatty acid content [45]. There are, however several drawbacks of acid catalysed biodiesel synthesis. Firstly, high molar ratios of alcohol to oil (approximately 5–50 fold, even higher in in situ experiments) are required to drive the reaction forward, secondly, reactions last longer than alkali-based catalysis and require temperatures of 60–90 °C. These conditions required additional heating and pressurized vessels to keep methanol in solution at temperature higher than 65 °C. Reports describing biodiesel production via acid catalysis are much more abundant than those of the alkali method [44].

2.2. Biodiesel-as an environment healthy fuel

Biodiesel is examined as ‘carbon neutral’ because all the carbon dioxide (CO_2) released during consumption had been sequestered from the atmosphere for the growth of vegetable oil crops [48]. The biggest advantages of biodiesel compared to many other alternative transportation fuels is that it can be used in existing diesel engines without modification, and can be blended in required ratio with petroleum diesel. Biodiesel performs like petroleum diesel, and also reduces emissions of particulate matter, carbon monoxide (CO), hydrocarbons and oxides of sulphur (SO_x) [48]. Emissions of oxides of nitrogen (NO_x) are, however, higher for biodiesel in many engines. Biodiesel eliminates the emissions of notorious black soot and the total particulate matter. Being highly biodegradable biodiesel also reduce emissions of air toxicants and carcinogens (relative to petroleum diesel). Various environmental benefits associated with 100% and 20% biodiesel blending, in term of pollutants emission reduction are given in Table 4. Using biodiesel will allow a balance to be sought between agriculture, economic development and the environment emissions as recently reviewed by Khan and his associates in 2009. According to OECD-FAO Agricultural Outlook 2010–2019, the production and usage of the biodiesel from different biodiesel sources in various countries is shown in Fig. 2a [49]. It is very much clear from the figure that the European Union dominates in the supply and use of biodiesel. Biodiesel has been in use in many countries such as United States of America, Malaysia, Indonesia, Brazil, Germany, France, Italy and other European countries. However, the potential for its production and application is much more. Fig. 2b shows the list of the top 10 biodiesel producing countries. From this figure, it can be judged that Malaysia is far ahead among the rest [modified from [50]].

3. Microalgae groups

Chen and his co-workers reported in 2009 that microalgae are microscopic single-celled or colonial photosynthetic organisms which are gaining interest very rapidly for industrial applications such as production of special chemicals & nutritional supplements. Microalgae are primitive organisms with a simple cellular structure and a large surface to volume body ratio, that provides them the ability to uptake large amount of nutrients. The mechanism of photosynthesis in microalgae is similar to that of higher plants but they are generally more efficient converters of solar energy because of their simple cellular structure [52,53]. Also as the algal cells grow in aqueous suspension, they have efficient access to water, CO_2, and other nutrients. Therefore microalgae are capable of producing 30 times the amount of oil per unit area of land as compared to terrestrial oilseed crops [2]. Microalgae are categorized in a variety of classes, mainly on the basis of their pigmentation, life cycle and basic cellular structure by several scientists [51]. The four most important group of algae in terms of abundance are represented in Table 5.

3.1. Microalgae an emerging source for biodiesel production

Microalgae are the unicellular algae that exist individually, or in chains or groups [4] that are one of the oldest living organisms and forms the base of the alimentary chain in the seas and rivers. There are more than 10^5 types of microalgae that are used to produce biodiesel only. It is reported [24] that in addition to being exceptionally diverse, they represent highly specialized group of organisms, which can adapt to various ecological habits. The idea of using algae for energy production has been around for over 50 years [54], the concept of using lipids derived from algal cells to produce liquid based arose recently [55]. The research of liquid fuel produced from microalgae was begun at middle 1980s in 20

Table 4
Biodiesel versus Diesel.
Source: Planning Commission of India, 2003.

Emissions	B100, pure biodiesel	B20, mixed biodiesel (20% biodiesel and 80% petroleum diesel)
1. Regulated emissions (%)		
Total unburned hydrocarbons	−93	−30
Carbon monoxide	−50	−20
Particulate matter	−30	−22
NO_x	+13	+2
2. Non-regulated emissions (%)		
Sulphates	−100	−20
Polycyclic aromatic hydrocarbons (PAHs)	−80	−13
Nitrated PAHs (NPAHs)	−90	−50
Ozone potential of HC	−50	−10
3. Life cycle emissions (%)		
Carbon dioxide	−80	–
Sulphur dioxide	−100	

(−): Less % of pollutant emission from biodiesel in comparison to 100% petroleum diesel.
(+): More % of pollutant emission from biodiesel in comparison to 100% petroleum diesel, i.e., only in the case of oxides of nitrogen (NO_x).

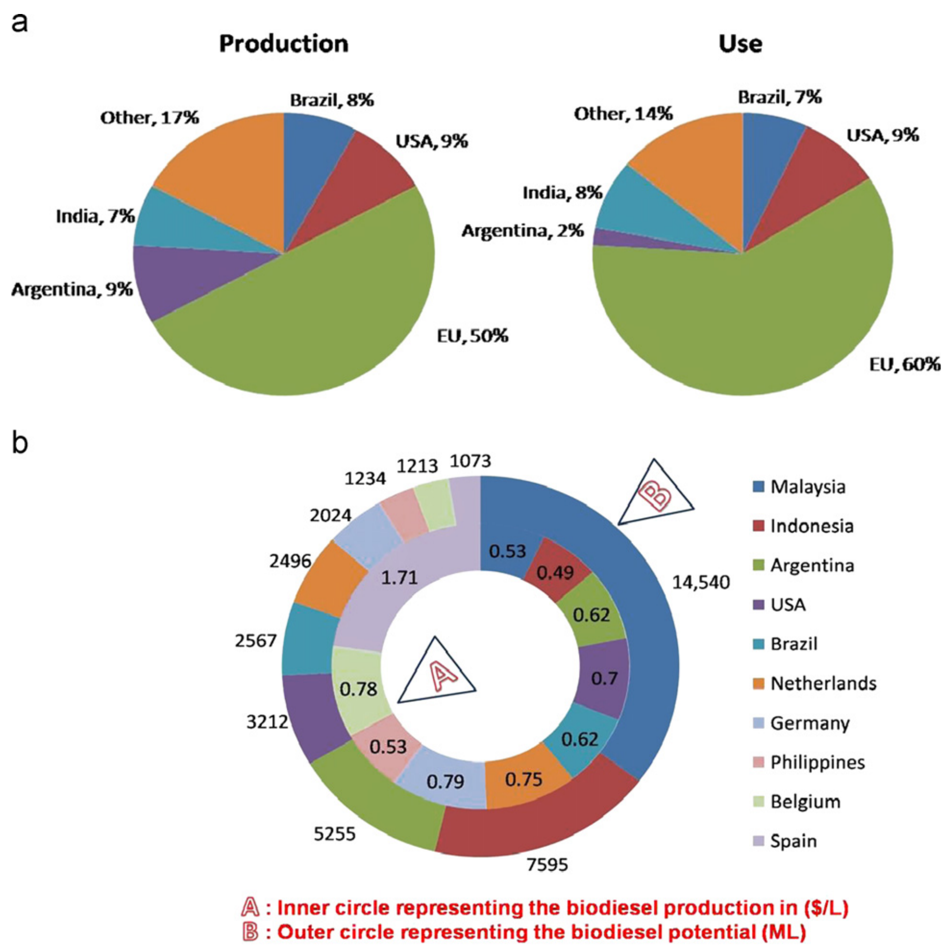


Fig. 2. (a): Percentage of biodiesel production and use by different countries of the world [49]. (b): Top 10 countries in terms of biodiesel potential and production (modified from [50]).

Table 5
The four most important group of algae in terms of abundance [130].

Sr. No.	Algae	Known species (near about)	Storage material	Habitat
1.	Diatoms (Bacillariophyceae)	100,000	Chyrsolaminarin (polymer of carbohydrates) and TAGs	Oceans, fresh and brackish water
2.	Green algae (Chlorophyceae)	8,000	Starch and TAGs	Freshwater
3.	Blue–green algae (Cyanophyceae)	2,000	Starch and TAGs	Different habitats
4.	Golden algae (Chrysophyceae)	1,000	TAGs and carbohydrates	Freshwater

centuries [16]. Aquatic biomass may represent a convenient solution, because it has a higher growth rate than terrestrial plants. Microalgae have been extensively studied so far, as they can grow both in fresh and salty waters and can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner. As they can be used to produce biofuel or even third generation biofuel. Compared with second generation biofuels, algal fuels have a higher yield: they can produce 30–100 times more energy per hectare compared to terrestrial crops. It has been found that fast-growing micro-algae can yield 1800–2000 gallons/(acre-year) of oil compare this with 50 gallons for soyabeans, 130 gallons for rapeseed and 650 gallons for palm oil [56]. Sean Milmo points out in his article [57] that oil from algae on 20–40 M acres of marginal land would replace the entire US supply of imported oil, leaving 450 M acres of fertile soil in the country entirely for food production. Quantifying the land use changes associated with intensive biofuel feedstock production relies upon many assumptions [2], but it is clear that the accelerated cultivation of terrestrial plant biomass for biofuels will have an exceptionally large land footprint as shown in Table 6.

Microalgae have been investigated for the production of a number of different biofuels including bioethanol, vegetable oils, biodiesel, bio-oil, bio-syngas, and bio-hydrogen. The production of these biofuels can be coupled with flue gas CO_2 mitigation, wastewater treatment, and production of high-value chemicals. Development in microalgal cultivation and downstream processing are expected to further enhance the cost effectiveness of the biofuel from microalgae [58]. Table 7 lists oil content of some of the microalgae on % dry weight basis.

Microalgae possess a high growth rate with duplicating the number of cells several times in a day [24,59] and also generate biomass in suitable reactors [24,59] many more times per unit area of land than growing agricultural crops that double in size over several days or weeks, or trees that grow on a timescale of years throughout the year. Numerous studies have argued that

Table 6
Comparison of estimated biodiesel production efficiencies from vascular plants and microalgae [131].

Biodiesel feedstock	Area needed to meet global oil demand (10^6 hectares)	Area required as a percent of total global land	Area required as a percent of total arable global land
Cotton	15,000	101	757
Soybean	10,900	73	552
Mustard seed	8,500	57	430
Sunflower	5,100	34	258
Rapeseed/Canola	4,100	27	207
Jatropha	2,600	17	130[a]
Oil palm	820	5.5	41
Microalgae (10 g m^3/day, 30% TAG)	410	2.7	21[b]
Microalgae (50 g m^3/day, 50% TAG)	49	0.3	25[b]

[a] Jatropha is mainly grown on marginal land.
[b] Assuring that microalgal ponds and bioreactors are located on non-arable land.

Table 7
Oil content in some microalgae [135].

Species	Oil content (% dry wt)	Reference
Botryococcus braunii	25–75	[2,132]
Chlorella spp.	28–32	[2]
Chlorella emersonii	63	[58]
Chlorella minutissima	57	[58]
Chlorella protothecoides	23	[58]
Chlorella sorokiniana	22	[58]
Chlorella vulgaris	40, 56.6	[58]
Cylindrotheca	16–37	[132]
Crypthecodinium cohnii	20	[132]
Dunaliella primolecta	23	[2]
Isochrysis spp.	25–33	[2]
Microtus Subterraneus	39.3	[58]
Monallanthus salina	> 20	[2]
Nitzschia laevis	69.1	[58]
Nannochloris spp.	20–35	[2]
Nitzchia spp.	45–47	[2,132]
Phyla. incise	62	[58]
Phaeodactylum tricornutum	20–30	[2]
Schizochytrium spp.	50–77	[2,132]
Tetraselmis sueica	15–23	[2]

biofuel (particularly biodiesel) production from algae is both economically and environmentally sustainable [17,60], although there have been some sceptical views of the long term viability and economics of biofuels from algae [61,62]. Microalgae possess a very simple cellular structure as compared to other oil crops. Depending on the species, their sizes can range from a few micrometers (μm) to a few hundreds of micrometers [4]. Chemical energy accumulated after the photosynthesis process is not diverted for the construction of complex structures but for the production of new cells. In addition, they are also potential source of biomass and specific products (e.g., lipids, pigments, antioxidants) [63]. Unicellular chlorophytic microalgae have been shown to be particularly tolerant to many wastewater conditions and very efficient at accumulating nutrients from wastewater [64]. Most current research on oil extraction is focused on the production of biodiesel from algal oil that itself is not significantly different from biodiesel produced from vegetable oils. Fig. 3 shows an outline of steps involved in production of biodiesel from Macro- or Micro-algae. The first step is the selection of an appropriate species with the relevant properties for the specific culture conditions and products. The culture conditions, including light, temperature, pH, air (carbon dioxide) and nutrient concentration, must be considered. Microalgae can be harvested using micro screens, sedimentation, centrifugation, flocculation or membrane filtration. The harvested biomass is then dried under vacuum to release water until it reaches a constant weight. The dried biomass is pulverized with a mortar and pestle before the oil is extracted through different methods like expeller/press, solvent extraction using chemicals and supercritical fluid extraction. After extraction, the oils are converted to biodiesel using one of the four primary methods like direct use and blending of raw oils, microemulsions, thermal cracking (pyrolysis) and transesterification.

The advantages of microalgae over higher plants as a source of transportation biofuels are numerous as recently reviewed by Khan et al in 2009:

1. Microalgae synthesize and accumulate large quantities of neutral lipids/oil [20–50% dry cell weight (DCW)] and grow at high rates (e.g., 1–3 doublings/day).
2. Oil yield per area of microalgae cultures could greatly exceed the yield of best oilseed crops.
3. Microalgae can be cultivated in saline/brackish water/coastal seawater on non-arable land, and do not compete for resources with conventional agriculture.
4. Microalgae tolerate marginal lands (e.g., desert, arid and semiarid lands) that are not suitable for conventional agriculture.
5. Microalgae utilize nitrogen and phosphorus from a variety of wastewater sources (e.g., agricultural run-off, concentrated animal feed operations, and industrial and municipal

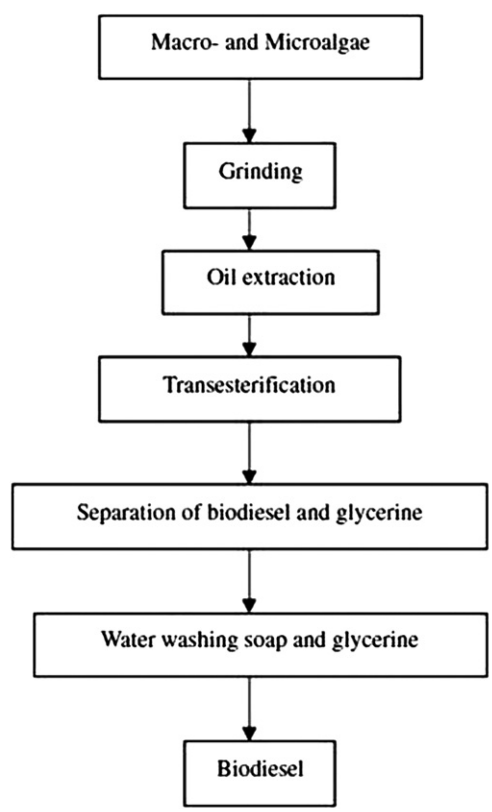


Fig. 3. Production of biodiesel from algae [133].

wastewaters), providing the additional benefit of wastewater bioremediation.

6. Microalgae sequester CO_2 from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing emissions of a major greenhouse gas. 1 kg of algal biomass requiring about 1.8 kg of CO_2 [65].
7. Microalgae produce value-added co-products or by-products (e.g., biopolymers, proteins, polysaccharides, pigments, animal feed and fertilizer) and do not need herbicide and pesticide.
8. Microalgae grow in suitable culture vessels (photobioreactors) throughout the year with higher annual biomass productivity on an area basis.

Disadvantages of microalgae for biofuel production is the low biomass concentration in the microalgal culture due to the limit of light penetration, which in combination with the small size of algal cells makes the harvest of algal biomasses relatively costly. The higher capital costs of the intensive care required by a microalgal farming facility compared to a conventional agricultural farm is another factor that impedes the commercial implementation of the biofuels from microalgae.

3.2. Contents of microalgae

Microalgae do not possess specialized structures, except for the presence of the pigments and photosynthetizers, they are composed mainly of carbohydrates, proteins and lipids and also serves as sources of essential vitamins (e.g., A, B1, B2, B6, C and E) although environmental factors, harvesting treatment and cell drying method determine their quantity [66]. Most of the microalgae have high protein content (that are used as food sources) and the carbohydrates are found mainly in the form of starch, glucose and other polysaccharides whose digestibility being high, can be used in dry form without any limitation [66]. Lipid content for use in food varies between 1–35% while that for biofuels lies between 20–80% [4,66] compared with 15–30% in vegetable oils, all on dry weight basis. As described by Satyanarayana et al in 2011 for using microalgae as a fuel, the algae should be of high calorific value and must be capable of growing in large volumes. Main contribution to the calorific value of cells is from their carbohydrate, protein and lipid content [3]. Microalgae grown under normal conditions possess calorific values in the range of 18–21 kJ g^{-1}, while the value for petrodiesel is 42 kJ g^{-1}. Some microalgae such as *Chlorella vulgaris* and *Chlorella emersonii* have been shown to grow in a 230 L pumped tubular photobioreactor in Watanabe's medium and a low nitrogen medium *C. vulgaris* can accumulate up to 58% lipid under low nitrogen conditions [3], whereas *C. emersonii* accumulates 63% lipids in small (2 L) stirred-tank bioreactors, which resulted in 29 kJ $g^{-1}$of calorific value [67] although the growth, productivity and lipid accumulation are yet to be determined at a larger scale. It is found [3] that this low nitrogen medium induces higher lipid accumulation in both algae, which increased their calorific value; the highest calorific value of 28 kJ g^{-1} was obtained with *C. vulgaris* with the biomass productivity of 24 mg dry wt $L^{-1}\,d^{-1}$ which was lower than that obtained with Watanabe's medium (40 mg dry wt $L^{-1}\,d^{-1}$). Nitrogen and phosphorus rich wastewaters have been proven suitable for microalgae growth [68] as the potential of microalgae to remove inorganic nitrogen, phosphorus, metal elements and other pollutants in the wastewaters has long been recognized [69]. Therefore, a wastewater based microalgae production has dual benefits of wastewater treatment and production of algal biomass with minimum external input of fresh water and nutrients [68]. It has been argued that microalgae biodiesel production in conjunction with wastewater treatment is the area with the most plausible commercial potential in the short term [70]. Wang et al. investigated the effectiveness of using digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp, and found that the total fatty acid content of dry weight was increased from 9.00% to 13.7%, along with removal of ammonia, total nitrogen, total phosphorus, and COD by 100%, 75.7–82.5%, 62.5–74.7%, and 27.4–38.4%, respectively [71]. Li et al. cultivated *Scenedesmus* sp. LX1 in secondary effluent, achieving high biomass yield (0.11 g L^{-1}, dry weight) and lipid content (31–33%, dry weight) [72].

3.3. Microalgal oil composition

It has been reported that algae produces more oil under stressed or unfavourable environment in comparison to optimal growth conditions [24]. During optimum growth conditions algae synthesize fatty acids principally for esterification into glycerol-based membrane lipids, which constitute about 5–20% of their DCW. Fatty acids include medium-chain (C_{10}–C_{14}), long-chain (C_{16}–C_{18}) and very long-chain ($\geq C_{20}$) fatty acid derivatives and during unfavourable conditions, many algae alter their lipid biosynthetic pathways towards the formation and accumulation of neutral lipids (20–50% DCW), mainly in the form of triacylglycerol (TAG). These TAGs primarily serve as a storage form of carbon and energy. After being synthesized, TAGs are deposited in densely packed lipid bodies located in the cytoplasm of the algal cell, although formation and accumulation of lipid bodies also occur in the inter-thylakoid space of the chloroplast in certain green algae [24]. The fatty acid composition of typical oil from microalgae is mainly composed of mixture of unsaturated fatty acids, such as palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3) along with small extent of saturated fatty acids, such as palmitic (16:0) and stearic (18:0) [40]. Microalgae have a very high oil production capacity which may reach up to 77% of their dry weight [2].

3.4. Applications of microalgae

First use of microalgae in China was about 2000 years back and the first concept of biogas production from microalgae started in fifties, and later it served as a source of different types of fuels, mainly liquid fuel (from *Botyrycoccus spp.*) [67], ethanol and methanol [73], gaseous fuel (methane) [74] as well as to produce hydrogen [75], a number of different application areas of microalgae have been identified [3,53,66,76,77]. These applications includes human and animal nutrition, cosmetics, high-value molecules such as fatty acids and pigments as well as natural dyes [78]. Many algae are also rich in omega-3 fatty acids, and as such, are used as diet supplements and components of livestock feed [79] along with the source of iodine, potassium, iron, magnesium, and calcium [80] that makes the microalgae as ideal sources of nutrients for functional foods preparation, food additive or in nutraceuticals. Now a days, interest in the development of active biomolecules from microalgae is in pipeline [81]. Microalgae have been used to fix CO_2 emitted from industrial waste gases [67] for wastewater treatments, as animal or human food and to produce numerous high-value bioactives [82]. Attempts have been made to develop filler materials using a microalgae (*C. vulgaris*) in various polymers such as polypropylene, poly vinyl chloride (PVC), polystyrene and polyethylene [2,3,77]. Certain species of algae can be used as an organic fertilizer, either in its raw or semi-decomposed form on land [83]. Some microalgae produce valuable by-products in the form of high value products like proteins, pigments, biopolymers and carbohydrates such as docosahexanoic acid and carotenoids including antioxidant substances for commercial or pharmaceutical purpose [84].

3.5. Lipids/fatty acids biosynthesis mechanism in microalgae

It is known that both inorganic carbon (CO_2) and organic carbon sources (glucose, acetate etc.) can be utilized by microalgae for lipids production. The components and contents of lipids in microalgal cells vary from species to species. The lipid classes basically are divided into neutral lipids (e.g., triglycerides, cholesterol) and polar lipids (e.g., phospholipids, galactolipids). Triglycerides as neutral lipids are the main materials in the production of biodiesel. The synthesis routes of triglycerides in microalgae may consist of the following three steps [18]: (1) the formation of acetyl coenzyme A (acetyl-coA) in the cytoplasm; (2) the elongation and desaturation of carbon chain of fatty acids; and (3) the biosynthesis of triglycerides in microalgae.

3.5.1. Formation of acetyl coenzyme A (acetyl-coA) in cytoplasm

The metabolism flux route on the utilization of CO_2 and glucose for the formation of acetyl-coA in microalgae is described by some scientists [85]. It is concluded that glyceraldehydes phosphate (GAP) is a key intermediate both for the two metabolism systems. The formation of acetyl-coA in photosynthetic reactions, light reactions, calvin cycle and synthesis, is located in chloroplast. GAP is withdrawn from Calvin cycle and exported to cytoplasm for consumption. After the export of GAP from chloroplast to cytoplasm, the flow of carbon is directed to the synthesis of sugars or oxidation through the glycolytic pathway to pyruvate. Sugars such as sucrose are the major storage products in the cytoplasm of plant cells. Some scientists [86] reported that glucose was easy to be stored as starch without prior conversion to GAP and then uptake by the chloroplast which suggested starch is the main storage formation for carbon source in *Chlorella spp*. So, one part of the exogenous glucose was directly converted to starch, and the remainder was oxidized through glycolytic pathway [18].

3.5.2. Elongation and desaturation of carbon chain of fatty acids

The elongation of carbon chain of fatty acids is mainly dependent on the reaction of two enzyme systems including acetyl-coA carboxylic enzyme (ACCE) and fatty acid synthase (FAS) in most organisms [18]. In the process of synthesis of fatty acids, acetyl- coA is the primer (Fig. 4a). The process of carbon chain elongation needs the cooperation with malonyl-coA, the substrate on which enzyme act are acetyl-ACP and malonyl-ACP. The C_{16}–C_{18} fatty acid thioester can be formed after several reaction steps. The formation of short carbon chain fatty acids is similar in the cells of advanced plants, animals, fungi, bacteria, and algae. For example, in the cell of green algae, the reaction routes of primer such as palmitoleic acid, oleic acid, linoleic acid, linolenic acid in fatty acid synthesis are similar to that in plant cells and yeast cells [87]. The desaturation of carbon chain of fatty acid occurs from C_{18} and further elongation of carbon chain takes place to produce long-chain fatty acids which are unusual in normal plant oils. Long-chain fatty acids (C_{20}–C_{22}) often exist in microalgae and the content varies from species to species [88]. Normally, short-chain fatty acids (C_{14}–C_{18}) which are the main components of biodiesel are majority of fatty acids in *Chlorella spp*., but high content of long-chain fatty acid and hydrocarbons exist in some specific species of microalgae. So, It is vital to choose proper microalgae species as materials of biodiesel production [18].

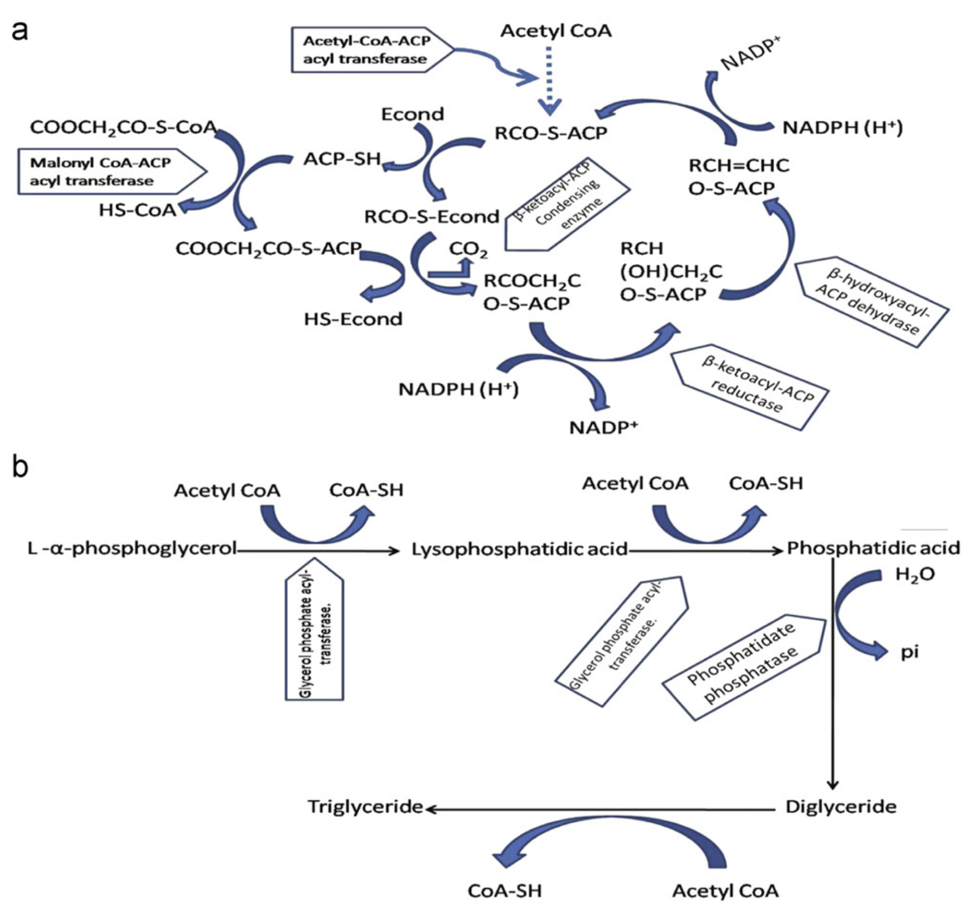


Fig. 4. (a) Schematic diagram of reaction process of the free fatty acid biological synthesis system (modified from [134]). (b) Schematic diagram of biosynthesis of triglyceride in microalgae (modified from [18]).

3.5.3. Triglycerides biosynthesis in microalgae

Microalgae are able to biosynthesize triglycerides to store substance and energy like other plants and animals. Generally, L-α-phosphoglycerol and acetyl-coA are two major primers in the biosynthesis of triglycerides. The L-α-phosphoglycerol mainly derives from phosphodihydroxyacetone which is the product of the glycolysis process. One of the hydroxyl in L-α-phosphoglycerol reacts with acetyl-coA to form Lysophosphatidic acid and later combines with another acetyl-coA to form phosphatidic acid. These two reactions are catalyzed by glycerol phosphate acyl-transferase. In the following steps, lysophosphatidic acid is hydrolyzed by phosphatidate phosphatase to form diglyceride which is then combined with the third acetyl-coA to complete the biosynthesis of triglycerides (Fig. 4b). The last reaction step is catalyzed by glyceryl diester transacylase [18].

3.6. Cultivation of microalgae in phototrophic mode for lipids production

The photosynthetic algae that requires light and CO_2 for their growth are classified as phototrophic algae. Microalgae can transform carbon dioxide from air and light through photosynthesis to convert into various forms such as polysaccharides, proteins, lipids and hydrocarbons. Microalgae have a number of advantages over higher plants including higher photosynthetic efficiency and growth rate [2]. In phototrophic culture, microalgae can be grown in open ponds and enclosed photobioreactors. Enclosed photobioreactor is more suitable for some microalgae which are readily contaminated by other microbes, except for some special microalgae which can survive well in extreme environments such as high pH (e.g., *Spirulina*) and high salinity (e.g., *Dunaliella*) or can grow very rapidly (e.g., *Chlorella*) in the open pond [18]. Due to the high cost in terms of operation and capital investment and the small scale production due to the complexity of bioreactor design compared to open pond system, it might not be economical to produce biodiesel on a large scale by using enclosed photobioreactors. As reported by Huang et al [18] Open pond system is perhaps more suitable for cultivating microalgae for biodiesel because of its relatively cheap operating cost. Commonly used procedure for cultivating microalgae is photoautotrophic mode of cultivation, because of its cost effectiveness it is preferred over heterotrophic mode. In photoautotrophic culture, the cells harvest light energy and use CO_2 as a carbon source, contributing to global CO_2 reduction. However, the photoautotrophic culture presents several limiting biomass production due to cellular self-shading that hinders light availability towards the end of growth. The low biomass concentration obtained in the photoautotrophic culture increases the biomass harvesting cost [89].

3.7. Cultivation of microalgae in heterotrophic mode for lipids production

Some algae are also capable of growing in dark and uses organic carbons such as glucose and acetate as energy sources and these are classified as heterotrophic algae. Microalgae can utilize light efficiently, phototrophic growth of microalgae is often slow because of light limitation at high cell densities on a large scale [90] or "photoinhibition", especially in sunny days [91]. Because of disadvantages associated with photoautotrophic cultivation, heterotrophic growth of microalgae is favorably considered [92]. Heterotrophic cultivation of microalgae offers several advantages over phototrophic cultivation including elimination of light requirement, good control of the cultivation process, and low-cost for harvesting the biomass because of higher cell density obtained [93]. In heterotrophic culture, both cell growth and biosynthesis of products are significantly influenced by various environmental and nutritional factors. Green microalgae *C. protothecoides* can grow photoautotrophically or heterotrophically but heterotrophic growth of *C. protothecoides* using acetate, glucose, or other organic compounds as carbon source results in much higher biomass as well as lipid content in the cells [94]. Heterotrophic microalgae might utilize other carbon sources such as ethanol, glycerol, and fructose depending on the microalgal species [18] [95]. The utilization of corn powder hydrolysate instead of glucose in heterotrophic culture greatly reduces the production cost of biodiesel in terms of economical significance. According to a study conducted by Huang and group [18] Nitrogen is an essential macronutrient in lipids production and complex nitrogen source might be superior to simple nitrogen source in heterotrophic culture of microalgae, as it will provide amino acids, vitamins and growth factors simultaneously. Industrial wastewater rich in nitrogen also can be used for the cultivation of microalgae. In the late 1940s, it was noted that nitrogen starvation is most influential on lipid storage and lipid fractions, and as a result of nitrogen starvation, the lipid content as high as 70–85% of dry weight was reported [96]. Nitrogen starvation might not always result in an increase in total lipid content but this may alter its lipid composition. Initial carbon to nitrogen (C/N) ratio in the medium also have a significant impact on the biosynthesis of lipids in microalgae. According to another study done by Miao and Wu by adding glucose as organic carbon source to the medium and the tremendous decrease of nitrogen source in the medium, crude lipid content up to 55.2% was achieved in heterotrophic *C. protothecoides*, which was about 3 to 4 folds that in photoautotrophic *C. protothecoides* [97]. Environmental factors also influence the growth and formation of fatty acids in microalgae. At low temperature high PUFAs are produced by algae to maintain cell membrane fluidity. Other reason may be the high level of intracellular molecular oxygen produced at low temperature which improves the activities of the desaturase and elongase involved in the biosynthesis of PUFAs [98]. But, the effect of temperature on cell growth and PUFAs production may not be always the same as mentioned above [23]. Hence, a specific and careful study of the individual microalgae is required. Salinity, pH, and dissolved O_2 are also important factors affecting the heterotrophic cultivation of microalgae [93]. Heterotrophically, grown microalgae usually accumulate more lipids than those cultivated photoautotrophically [97]. In contrast to photoautotrophic culture, heterotrophic culture can be performed in conventional microbial bioreactors. Thus it is much easier to alter conditions to improve the yield of biomass and reduce the cost of microalgal biomass production [18]. Recently, Zhao et al. reported that some microalgal species can conduct mixotrophy in the culture containing both inorganic and organic substrates which are simultaneously assimilated, with both respiratory process and photosynthesis occurring concurrently [99]. According to their study, the growth rate of mixotrophic culture is the sum of the photoautotrophic growth and heterotrophic growth. The mixotrophic growth of some microalgae produced 3–10 times more biomass yields as compared with phototrophy [100]. The biodiesel from heterotrophic microalgal oil could be a competitive alternate to conventional diesel fuel [15].

4. Future research and development aspects regarding microalgae and algal biofuel production

There is a growing interest to develop cost-effective processes and to enlarge their application areas based on various advantages mentioned earlier in this article. These include bulk biological chemicals and rapidly growing biofuel industries. Apart from regular activities of isolation and characterization, culturing still remains a niche area needing continued R&D efforts towards cost-effective technologies [2,24]. Research efforts should be made

towards additional organisms which may possess unique mechanism for efficient production of lipid/oil. Innovative development of large-scale culture systems through proper selection of algal strains that lead to high and sustained growth rates of oil-rich biomass have to be studied further [24]. According to a recent review of Satyanarayana and his co-workers in 2011 production of higher biomass yield through genetic engineering to increase the photosynthetic efficiency or to produce higher yields of oil, stability of such strains, identification of new strains capable of growing faster at high cell densities, increasing the growth rate of biomass and its oil content, reduction of photooxidation susceptibility which damages cells, identification of factors including biochemical triggers and environmental that enhances the oil content are some of the issues needing greater attention [2,24,76].

An important issue is to achieve cost-effective photobioreactors with high efficiency, to achieve maximum productivity with minimum operation costs. Downstream processing is one of the major issues in microalgal biotechnology, which includes separation of biomass and concentration of microalgae culture. Development of cheaper and energy conserving processes [24,82] includes genetic modification and engineering of algal strains from dilute cultures with optimum photosynthesis and product formation [76]. Also, development of economical, quick and efficient processes for harvesting and de-watering of biomass depending on the end use is another area of interest for R&D [24,76].

Another issue is the higher cost of microalgal fuel (biodiesel) production. Some of the methods to eliminate problem may be by (i) resorting production strategy to integrated biorefinery, where useful products are produced using every component of biomass [2], (ii) identifying high value products particularly big molecules type based on specific microalgae [2] or broadening commercially viable product range such as nutraceuticals based on highly productive heterotrophic cultures [24] and (iii) sale of generated excess power [2]. Lipid extraction is also an important step [27] which must be addressed. Although biofuel is considered environmentally less harmful than diesel fuel, its eco-compatibility depends on method of its production, use and trade [101]. These in turn determine its economic, environmental and social aspects since not much is reported on its eco-toxicological information of non-regulated emissions, effluents generated during its production and on its water-soluble fractions (WSF) as reviewed by Satyanarayana and associates in 2011. Therefore studies are needed including modeling to look into toxic effects caused by this fuel particularly the WSF of biofuels, some attempts towards these have recently initiated [101].

Algal-biofuel research originated in 1979, when the U.S. Department of Energy (DOE) initiated a research program called the Aquatic Species Program (ASP) and it was closed in 1995 due to a budget reduction. The high growth rates, reasonable growth densities and high oil contents have all been the areas to invest significant capital to turn algae into biofuels. Apart from this a number of hurdles to overcome like how and where to grow these algae, to improve oil extraction and fuel processing. The algal biofuels production chain shows that the major challenges that include strain isolation, nutrient sourcing and utilization, production management, harvesting, coproduct development, fuel extraction, refining and residual biomass utilization. Improved engineering will make a significant impact on algae biofuel production. These improvements include efficient strategies for nutrient circulation and light exposure, and have been reviewed elsewhere [102,103]. There are significant challenges for engineers and biologists to either design cheap photobioreactors (PBRs) for large-scale deployment, or to combine forces to develop species that grow efficiently in low-cost open systems [104]. PBRs have advantages over open systems as they can more easily maintain axenic cultures, controlled growth environments, which may lead to increases in productivity and decrease in contamination; however, contained systems are challenged by efficiencies in gas exchange and a requirement for supplemental cooling. Regardless of the growth strategy employed, substantial improvements over current technologies for the growth, harvesting and extracting oil from algae need to be made, and coordinated efforts will be needed to couple engineering advances with improved production strains [105]. Oil extraction is another challenge and there are three major strategies for extracting oil from algae: oil press/expeller, hexane extraction, and supercritical CO_2 fluid extraction [106]. These technologies have been successfully demonstrated but are relatively expensive, either in terms of equipment needed or energy required to extract the oil. Once extracted, because crude algae oil is chemically similar to crude fossil fuel oil, the engineering challenges associated with algae oil conversion to usable liquid fuels are similar to those already well managed by petroleum companies, although improved catalysts will be required to improve gasoline production from bio-oil [107]. Because of these similarities, it seems reasonable to assume that collaborations between algae production companies and major oil companies are likely, since these companies have extensive experience maximizing downstream processing efficiencies [105]. The research and development efforts can be categorized into several areas [108]:

1. Increasing oil content of existing strains or selecting new strains with high oil content;
2. Increasing the growth rate of algae;
3. Developing robust algal-growing systems in either open-air or enclosed environments;
4. Developing co-products other than oil;
5. Using algae in bioremediation; and
6. Developing an efficient oil-extraction method.

Many challenges have hindered the development of biofuels technology from microalgae to become commercially viable. Among them, and based on recent literature, the most important ones as reported in review of Ribeiro and da Silva [109] are:

- The selection of species must balance the requirements for biofuel production and extraction of valuable by products [110].
- Continuous development of production systems so that we can achieve greater photosynthetic efficiency via microalgae [111].
- Developing techniques for growing a single species, reducing losses due to evaporation and diffusion of CO_2 [112].
- Choosing algae strains that require freshwater to grow can be unsustainable for operations on a large scale and exacerbate freshwater scarcity [113].
- Few commercial cultivating "farms," so there is a lack of data on large-scale cultivation [114].
- Impossibility of introducing flue gas at high concentrations, due to the presence of toxic compounds such as NO_x and SO_x [115].
- Current harvest and dewatering are still too energy intensive [116].
- Some recent life cycle analyses (LCAs) project algae biofuels as having poor energy or greenhouse gas benefits [117].
- Another disappointment that will likely arise is the scarcity of sites with favourable climate, land, water, and CO_2 resources, all required in one place [117].
- CO_2 supply is relatively expensive, due to high capital and operational costs for piping CO_2 to, and transferring it into, the ponds [117].

The method to achieve these goals is to genetically and metabolically alter algal species & to develop new growth technologies or to improve existing ones so that the same goals

listed above are met. Large scale cultivation of algae for biodiesel production is still in the research & development phase. Currently it is too expensive to be commercialized. The long term potential of this technology can be improved by the following approaches [118].

- Cost saving growth technologies of oil-rich algae should be identified and developed.
- Integrated bio-refineries can be used to produce biodiesel, animal feed, biogas and electrical power thereby reducing the cost of production.
- Enhancing algal biology by genetic modification and metabolic engineering has the greatest impact on improving the economics of microalgae biodiesel.
- Area efficient techniques to capture CO_2 from industrial power plants need to be identified.
- Recycling of nutrients from municipal sewage and industrial waste waters are required to reduce the demand of fertilizers to grow algae.
- Economics of microalgae production can be improved by additional revenues from waste water treatment and greenhouse gas abatement.

5. Current economic assessment of biofuel production from microalgae

On the basis of current technology The cost estimates of algal-based biofuels ranges from US $300–2600 per barrel and we need to overcome the technical hurdles to improve this cost price [105]. Improvements can come by improving growth strategies and engineering, and can also be done by optimizing the entire organism use. From the present small production facilities, it is difficult to extrapolate the final price of a barrel of algae oil during large scale production. Most analysts do not predict full parity with petroleum in the near future. More likely, the initial selling point of algal fuel will be approximately twofold higher than petroleum, but the environmental costs will be substantially lower than our current strategy of depending on fossil fuels. Certainly, a premium price is warranted for clean fuels (fuels that have a 50% lower CO_2 cradle-to-grave footprint than petroleum); however, estimated costs of a barrel of algae-based fuel using current technology is US$300–2600, compared with $40–80 (2009) for petroleum [2,119–121]. According to some estimates for a barrel of algae oil in specific regions reach as low as $84 [122]. The higher dollar estimates are more common and exclude algal oil from the current liquid fuel market. The production cost of algal oil depends on many factors, such as yield of biomass, oil content, scale of production systems, and cost of recovering oil from algal biomass. Currently, algal-oil production is still far more expensive than petroleum diesel fuels. Chisti in 2007 estimated the production cost of algae oil from a photobioreactor with an annual production capacity of 10,000 t per year [2]. Assuming the oil content of the algae to be approximately 30 percent, Chisti in 2007 and some other scientists [2,123] determined a production cost of $2.80 per liter ($10.50 per gallon) of algal oil and it did not included the costs of converting algal oil to biodiesel, distribution and marketing costs and taxes. If producers can use fatter microalgae (e.g., with at least 60% oil content) that grow faster, then they can reduce both the size and footprint of the biofuel plant can be reduced upto by as much as half that will provide a significant reduction in capital and operating costs. Based on several available reports [124], the current estimated costs of production of microalgal biodiesel lie between US $9 and $25 per gallon in ponds, and within the range US $15-$40 in photobioreactors. Since microalga production systems are a complex combination of several subsets (i.e., production, harvesting, extraction and drying systems), the crucial step is to reduce the cost associated with the process. The future of the algal oil to become economic source of biofuel highly depends on the petroleum oil price [108]. Some scientists [2] used the following equation to estimate the cost of algal oil where it can be a competitive substitute for petroleum diesel:

$$C_{\mathrm{Algal\ oil}} = 25.9 \times 10^{-3} C_{\mathrm{Petroleum}}$$

where, $C_{\mathrm{Algal\ oil}}$ is the price of microalgal oil in dollars per gallon and $C_{\mathrm{Petroleum}}$ is the price of crude oil in dollars per barrel

This equation assumes that algal oil has roughly 80 percent of the caloric energy value of crude petroleum. For example, with petroleum priced at $100 per barrel, algal oil should cost no more than $2.59 per gallon in order to be competitive with petroleum diesel.

According to the final report submitted by the Seed Science Ltd. to the British Columbia Innovation Council in January, 2009 [125], extensive review of the literature and information obtained from industry insiders resulted in the identification of the cost parameters for expected biomass yields, algae oil content, capital, labour and operational costs. A thermodynamic model was developed that uses hourly solar insulation and temperature values in base case to predict maximum biomass yields for the two phototrophic technologies. Using this model, the resulting cost per litre of algae oil were determined under four different scenarios like a) Unheated raceway (run year round) b) Heated raceway (run year round) c) Unheated raceway (run April to September) d) Unheated raceway (run April to September with half the capital costs of scenario c). The results of the economic analysis for each scenario along with photobioreactor, fermentor and canola was expressed both in terms of cost ($) per kg of biomass produced and in cost per liter of oil produced. The cost per litre of algae oil was $24.60, $17.60, $14.44, $8.24, $7.64, $5.87, $2.65, $2.58, $1.54 and $0.88 for photobioreactor (base case), photobioreactor (35% oil), raceway (base case), raceway (heated), raceway (summer only), photobioreactor (ethanol), raceway (summer with 25% oil and 50% capital cost), fermentor (base case), fermentor (100 g L^{-1}, 60% oil) and canola, respectively. The cost per kg of algal biomass was found to be $7.32, $7.32, $2.66, $1.57, $1.54, $1.46, $1.11, $1.02 and $0.35 for photobioreactor (base case), photobioreactor (35% oil), raceway (base case), raceway (heated), fermentor (base case), raceway (summer only), fermentor (100 g L^{-1}, 60% oil), raceway (summer with 25% oil and 50% capital cost) and canola, respectively. The base case costs for the three different production processes are illustrated in Table 8. Raceways have total production costs of $14.44 per litre of algal oil. Majority of this cost involves capital (49%) and labour costs (27%). However, operational costs such as power and fertilizer are also substantial (25%). Photobioreactors have a higher production cost of $24.60 per litre of algal oil. In raceways, majority of this cost is capital (63%). Fermentors have the lowest production cost of $2.58 per litre of algae oil. This time the majority of the cost is operational (78%) - mainly from the power (31%) and the organic carbon substrate (23%). Even under the optimistic scenarios, currently, none of the processes examined in this study can achieve price parity with fossil fuels. Cost effective algal biomass production through fermentation still requires significant R&D to generate greater yields and oil content. Recently, Resurreccion et al. used combined life cycle assessment and life cycle costing approach to evaluate open pond systems and horizontal tubular photobioreactors for the cultivation of freshwater or brackish-to-saline water algae. Based on the life cycle assessment, open pond systems have lower energy consumption

Table 8
Base case costs for the three different production processes [125].

	Raceway		Photobioreactor		Fermentor	
Initial investment ($\$L^{-1}$)	52		111		2	
Production cost	($ per liter of oil produced)					
Labour cost	$4.03	26.69%	$2.96	11.90%	$0.29	10.88%
Other production cost	$3.71	24.59%	$6.37	25.59%	$2.06	78.45%
Capital cost	$7.35	48.71%	$15.56	62.50%	$0.28	10.66%
Total cost	$15.09		$24.89		$2.63	
Credit from the sale of algae cake*	$0.65		$0.29		$0.05	
Net total cost	$14.44		$24.60		$2.58	
Lipid content	15%		25%		50%	
Cost per kg of algae	$2.66		$7.32		$1.54	

* Including revenues from selling the algae cake after oil extraction.

and greenhouse gas emissions than photobioreactors; e.g., 32% less energy use for construction and operation. According to the life cycle costing approach, all four systems are currently financially unattractive investments, though open pond systems are less so than photobioreactors. Brackish-to-saline water algae species deliver better energy and GHG performance and higher profitability than freshwater species in both OPs and PBRs [126]. Microalgal oils have potential to completely replace petroleum as a source of hydrocarbon feedstock for the petrochemical industry. For this, microalgal oil needs to be produced at a price, which is less than the price of crude oil [127]. It has been shown that it is scientifically and technically possible to derive the desired energy products from algae in the laboratory. The question lies, however, in whether it is a technology that merits the support and development to overcome existing scalability challenges and make it economically feasible [113]. Economic viability is believed to be currently the main hurdle to overcome for this technology. Current costs associated to both the state of the science and technologies are sizeable and represent a main factor working against development. In this perspective, algal biofuels are generating substantial awareness in many countries. In the United States, they may contribute to achieve the biofuel production targets set by the Energy Independence and Security Act of 2007. Likewise, in the European Union, they may assist to the achievement of goals established in the recent renewables directive. In order to address the technical-economic barriers to the further development of this type of bioenergy, it is thus necessary to contribute with a study that incorporates biomass feedstock availability assessment, production, management, and harvesting in support of the up scaling of this promising technology [109]. Different bioenergy pathways are at various stages of maturity. Several technologies' most critical need is to demonstrate efficiency at the appropriate scale-pilot plants, pre-commercial demonstration or full industrial scale [109]. By 2020, the contribution to the EU energy mix from cost competitive bio-energy used in accordance with the sustainability criteria of the new renewable energy sources (RES) directive could be at least 14%. It can be conclusively established that, the economics of biodiesel production can be improved by advancing the production technology. As the yield per acre of microalgal oil is already far more than the yield of oil from palm and jatropha plant [128]. The by-products obtained after the production of biodiesel from algae oil is a high value protein that can be used as cattle feed and as a soil additive in mulching as a biofertilizer [129].

6. Conclusion

Recently, finding new energy resources to replace petroleum has been a current & emerging topic of interest worldwide. Algal biofuel is an ideal candidate which eventually could replace petroleum-based fuel due to several reasons like high oil content, high production, less land requirement, etc. Microalgae are the only biofuel source that could be grown without competing with agricultural land and contain a large percentage of oil, with the remaining parts consisting of large quantities of proteins, carbohydrates, and other nutrients [53]. This makes the residue after oil extraction attractive for use as animal feed or in other value-added products. Based on several available reports [124], the current estimated costs of production of microalgal biodiesel lie between US $9 and $25 per gallon in ponds, and within the range US $15–$40 in photobioreactors. Since microalga production systems are a rather complex combination of several subsets (i.e., production, harvesting, extraction and drying systems), reducing the associated number of steps is thus crucial to lower cost. Whether algal oil can be an economic source for biofuel in the future is still highly dependent on the petroleum oil price. In order to maneuver the technical-economic barriers for future development of bioenergy from microalgae, it is necessary to conduct the studies that targets at biomass feedstock availability assessment, production, management, and harvesting in support of the up scaling of this promising and advance technology.

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